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〔研究ノート〕

# Genome-wide approach for screening of functional genes causing overexpression-mediated growth inhibition in fission yeast *Schizosaccharomyces pombe*

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**Key words**: fission yeast, genome analysis, overexpression

The fission yeast *Schizosaccharomyces pombe* is an excellent model system for functional and comparative studies of eukaryotic cell processes. The genome sequence has already determined [1], but the functions of most predicted 4,950 ORFs are still unknown. In the classic genetic strategy, the recessive (loss of function) mutant cells were analyzed to identify functional genes. However, the strategy is not adequate for genome-wide search of functional genes. Here, we report a simple approach that permits the rapid genome-wide screening of functional genes of the fission yeast *Schizosaccharomyces pombe* by using the dominant genetic strategy.

Standard fission yeast techniques and media were employed [2]. *S. pombe* strains expressed each 3′-tagged ORF constructed as described below were used in this study. Each ORF was cloned into *S. pombe* expression vector pDUAL-FFH1c, a plasmid that enables both multicopy maintenance and chromosomal integration of the transgene [3]. The cloned ORFs were expressed under the control of the thiamine-regulatable *nmt1* promoter [4], because this promoter allows controlled expression of the cloned ORFs and reduces expression of toxic ORFs. The 3′-tagged ORF plasmids were integrated individually into the *leu1* locus of the *leu1-32* strain AM2 [5]. The tagged ORF expression is induced in MM medium (absence of thiamine) and repressed in SD medium (presence of thiamine). The cells were initially grown on SD liquid medium [6.7% yeast nitrogen base without amino acid (Difco), 2% glucose] overnight at 30℃. To allow expression genes driven by the *nmt1* promoter, the cells washed twice with distilled water were subsequently suspended in MM liquid medium and grown at 30℃ for 24 h. For selection of the genes causing overexpression-mediated growth inhibition, cells adjusted to $1OD_{600}$ were spotted onto MM solid medium and grown at 30℃ for 2d. For investigation of effect of overexpression shut-off of the genes on growth recovery, after incubation at 30℃ for 24 h in MM liquid medium to allow expression genes driven by the *nmt1* promoter, cells adjusted to $1OD_{600}$ were spotted onto MM solid medium containing 1.67 mg/ml of thiamine and grown at 30℃ for 2 d.

The cells were initially grown on SD solid medium at 30℃ for 2-3 d. To allow expression genes driven by the *nmt1* promoter, they were subsequently suspended in MM liquid medium and grown at 30℃. After 16 h, the cells were transferred to fresh MM medium to

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dilute out thiamine and sequentially grown at 30℃ for 10 h. Staining of the cells with DAPI, Calcofluor white (fluorescent brightener 28; Sigma), or BODIPY® FL phallacidin (Molecular Probes) was performed as described [6, 7]. Images were captured using a Zeiss Axio Imager fluorescence microscope equipped with a Plan Apochromat x63 lens and a Zeiss AxioCam HRm.

One hundred and sixty-nine strains predicted to be overexpression sensitive strains in the preliminary screening (data not shown) were used for screening of overexpression sensitive strain in this study. Figure 1 shows the colonies grown on MM solid medium. The 169 strains examined were grouped based on the growth point. '3 point' means that the colony grew as well as that of mock (control; ORF was not integrated to expression vector), '2 point' means that the colony did not grow as well as mock, '1 point' means that the colony hardly grew, and '0 point' means that the colony was not form at all. The growth point of the strain is a sum of 'point' at each dilution as shown in Fig. 1. Out of 169 strains examined, 20 strains showed growth point 0 (e.g. 30/H11 in Fig. 1), 57 strains were growth point 1 (e.g. 19/A05 in Fig. 1), 36 strains were growth point 2 (e.g. 19/A12 in Fig. 1), 22 strains were growth point 3 (e.g. 05/A03 in Fig. 1), 14 strains were growth point 4 (e.g. 11/C07 in Fig. 1), 3 strains were growth point 5 (e.g. 51/A09 in Fig. 1), 8 strains were growth point 6 (e.g. 20/E09 in Fig. 1), 4 strains were growth point 7 (e.g. 39/G04 in Fig. 1), 2 strains were growth point 8 (e.g. 12/G04 in Fig. 1), and 3 strains were growth point 9 (e.g. 45/F11 in Fig. 1). No strain was classified into growth point 10, 11 or 12. Consequently, the 169 ORFs examined here caused growth inhibition to some extent by overexpression.

To determine whether cells of the 20 strains of growth point 0 under the overexpression conditions were dead or not, we examined the effect of a shut-off of their cloned genes expression. Figure 2 shows the colonies of the representative 2 strains (03/E11 and 30/H11) grown. The colonies on MM solid medium containing 1.67 mg/ml of thiamine grew as well as that of mock without any ORF, while the colonies on MM solid medium in the absence of thiamine were not formed at all. It indicates that the growth inhibition

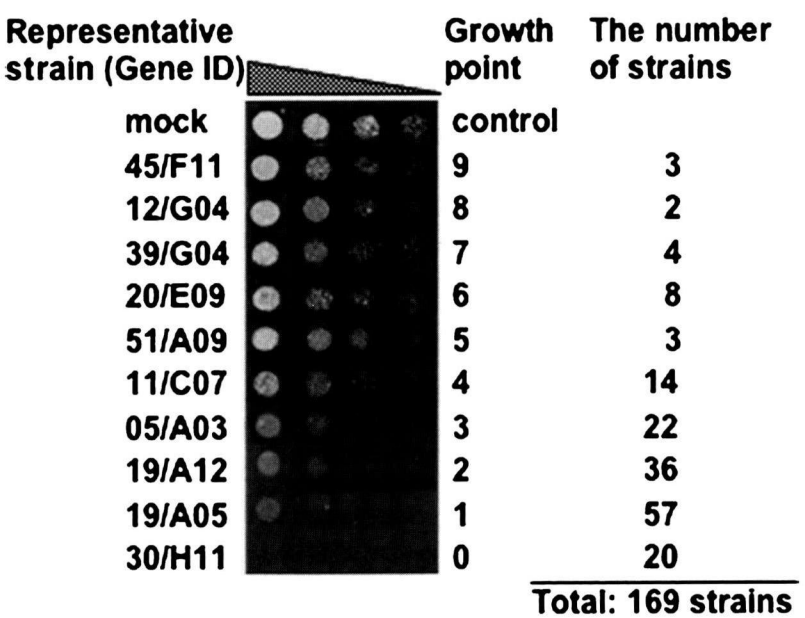


**Fig. 1.** Screening of the gene causing overexpression-mediated growth inhibition. Strains (cell density = 1$OD_{600}$) were spotted at 1 : 10 dilutions onto MM solid medium in the absence of thiamine. The plates were incubated at 30℃ for 2 d. The growth point is described in the text.

caused by overexpression was recovered by reduced expression of genes driven by the *nmt1* promoter. It seems likely that the 20 strains of growth point 0 which did not grow at all in the absence of thiamine were still capable of proliferating after releasing the growth arrest.

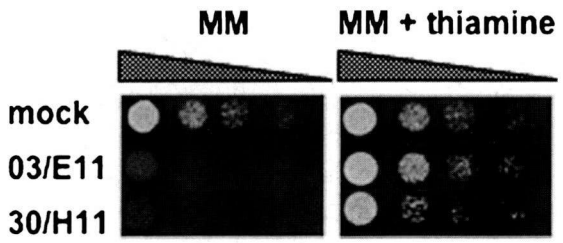


**Fig. 2.** Effect of overexpression shut-off on recovery of growth. After incubation at 30℃ for 24 h in MM liquid medium to allow expression genes driven by the *nmt1* promoter, strains (cell density = $1OD_{600}$) were spotted onto MM solid medium containing 1.67 mg/ml thiamine at 1 : 10 dilutions. The plates were incubated at 30℃ for 2 d.

The phenotype of cells of the 20 strains of growth point 0 caused by the overexpression was investigated by Nomarski and fluorescence microscopy. Overexpression of the genes of the 19 strains resulted in abnormal cell shapes, such as elongated, round or swollen cells (Table 1). The representative microphotographs are shown in Fig. 3. The elongate cells (7 strains in Table 1) were characterized by abnormal septa; no septum, two or more septa per cell and/or irregular shape. The round cells (4 strains in Table 1) were characterized by abnormal F-actin localization and/or irregular nuclear shape. The swollen cells (8 strains in Table 1) were characterizes by abnormal F-actin localization and/or irregular shape of nuclei and septa. Overexpression of the gene of the remaining

**Table 1** Genes causing growth inhibition that the colony did not grow at all induced by overexpression.

| | morphology | Gene ID (strain)[1)] | Gene Name[2)] | Gene Product[2)] | Systematic Name[2)] |
|---|---|---|---|---|---|
| elongate | thick septa | 21/H06 | *alp7* | predicted coiled-coil protein | SPAC890.02c |
| | nuclear division immature | 30/C01 | *crm1* | nuclear export receptor Crm1 | SPAC1805.17 |
| | branched, irregular septa | 30/H09 | *peg12* | mitotic spindle protein | SPAC3G9.12 |
| | maltisepta | 30/H11 | SPAC56F8.02 | AMP binding enzyme | SPAC56F8.02 |
| | thick septa, aberrant nuclei | 38/C08 | *alp4* | spindle pole body component | SPBC365.15 |
| | irregular septa, branched | 38/E07 | *kap95* | karyopherin | SPAC1B1.03c |
| | no septa | 47/C06 | *srs2* | UVRD helicase | SPAC4H3.05 |
| round | lost polarity | 01/F10 | *mrp10* | mitochondrial ribosomal protein subunit | SPAC24C9.13c |
| | lost polarity | 05/D08 | *rim1* | single-stranded DNA-binding protein | SPAC2F3.04c |
| | lost polarity, aberrant nuclei | 12/B09 | SPAC8F11.06 | hypothetical protein | SPAC8F11.06 |
| | lost polarity | 36/E12 | *met9* | methylenetetrahydrofolate reductase | SPAC56F8.10 |
| swollen | lost polarity | 03/E11 | *cam2* | myosin I light chain Cam2 | SPAC29A4.05 |
| | thick septa | 07/E12 | SPCC126.11c | RNA-binding protein | SPCC126.11c |
| | irregular septa | 11/F06 | *tom20* | mitochondrial outer membrane translocase complex | SPAC6F12.07 |
| | aberrant nuclei | 20/F08 | SPAC10F6.17c | serine-threonine protein phosphatase | SPAC10F6.17c |
| | aberrant nuclei | 22/C05 | *tim44* | mitochondrial import inner membrane translocase subunit | SPBC14C8.02 |
| | irregular septa | 28/G01 | *tea4* | src (SH3) homology domain | SPBC1706.01 |
| | thick septa | 35/E08 | *tnr3* | thiamine pyrophosphokinase (thiamine kinase) | SPAC6F12.05c |
| | thick septa, nuclear condensation | 48/F08 | *nup146* | nucleoporin | SPAC23D3.06c |
| normal | cell cycle arrested | 34/C09 | *tor2* | phosphatidylinositol kinase | SPBC216.07c |

[1)], Gene ID (strain number) in this study.
[2)], From *Schizosacchromyces pombe* GeneDB [http://www.genedb.org/genedb/pombe/index.jsp].

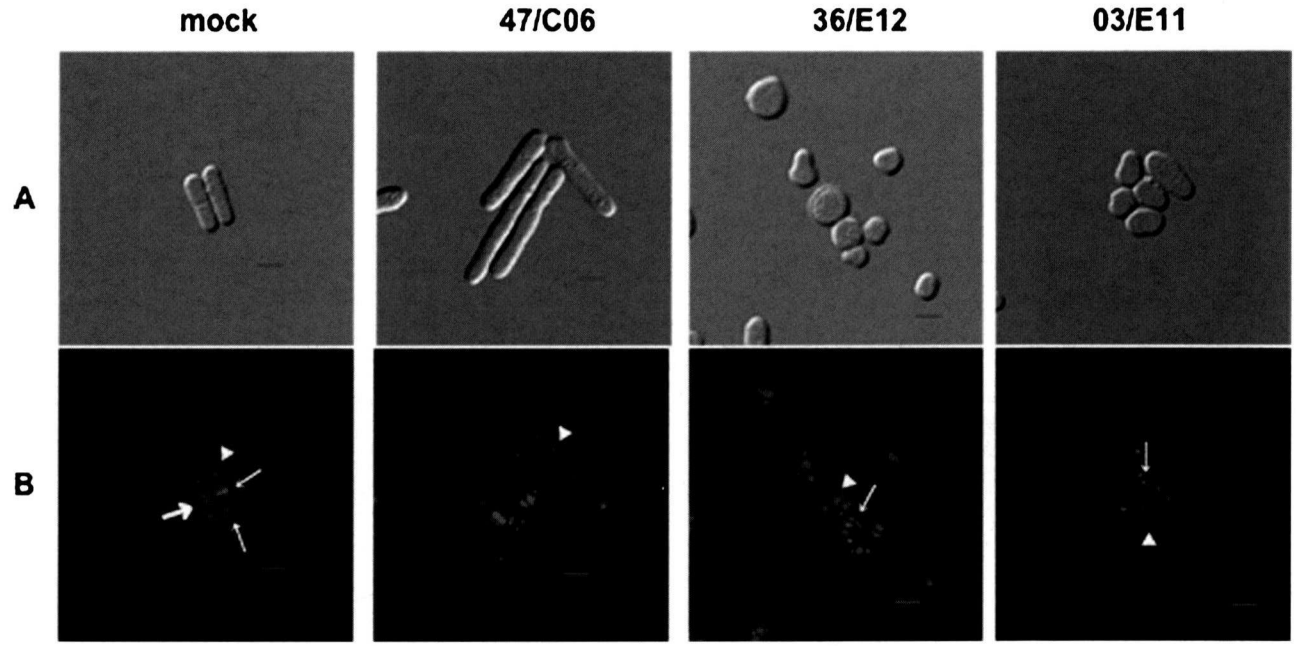


**Fig. 3.** Micrographs of cells containing an integrated *nmt*: ORF construction growing in the absence of thiamine at 30℃ for 10 h. (A) Nomarski photomicrographs. (B) Fluorescence photomicrographs. Cells were stains with DAPI (blue), Calcofluor white (blue), BODIPY® FL phallacidin (green). Arrowheads, large arrows and small arrows indicate the nuclei, septa and F-actin, respectively. Bar represent 5 $\mu$m.

strain (34/C09) showed normal cell shape (image data not shown), but seemed to arrest the cell cycle in G1.

As the correct positioning of the nucleus, septum and actin is very important organelles for growth in fission yeast, it is conceivable that the twenty genes inducing overexpression-mediated abnormal phenotypes are functional genes for the regulation of proliferation. As for the gene 21/H06, the abnormal phenotype of the elongate cell with thick septa induced by overexpression (Table 1) suggests that the gene is important for cell division during mitosis. Experimentally, the function of the gene 21/H06 (*alp7*) is determined to involve in the regulation of mitotic progression [8]. As for the gene 30/C01, the abnormal phenotype of nuclear division immature in the elongate cells induced by overexpression (Table 1) suggests that the gene is important for chromosomal region maintenance. Experimentally, the function of the gene 30/C01 (*crm1*) is determined to involve in chromosome compaction and nuclear positioning [9]. As for the gene 38/C08, the abnormal phenotype of the elongate cell with thick septa and aberrant nuclei induced by overexpression (Table 1) suggests that the gene is important for cell division and chromosome segregation during mitosis. Experimentally, the function of the gene 38/C08 (*alp4*) is determined to involve in G1 progression (required), spindle assembly checkpoint and cytokinesis [10]. As for the gene 38/C08, the cells appeared arresting in the G1 phase during the mitotic cell cycle induced by overexpression (Table 1) suggests that the gene is important for cell differentiation, because cell-cycle arrest in pre-Start G1 is a prerequisite for cell differentiation in fission yeast [11, 12]. Experimentally, the function of the gene 34/C09 (*tor2*) is determined to involve in starvation response (required) and stress response [13]. As described above, the phenotype induced by overexpression in this study may be useful for determination of the gene function. It is therefore supposed that the strategy using the genome-wide overexpression screen is an alternative to the phenotypic screen of the recessive mutant cells

for analysis of functional genes. Indeed, important regulatory genes are not always essential for cell growth, which can be analyzed by recessive mutations. Thus, the genome-wide dominant genetic search in this study will allow identifying such the functional genes regulating proliferation in fission yeast.

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# 過剰発現による増殖阻害を指標とした分裂酵母 *Schizosaccharomyces pombe* 機能遺伝子の網羅的探索

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高等生物の新規遺伝子の機能予測を目的としたゲノム塩基配列の相同性解析のための極めて優れたモデル生物である分裂酵母 *Schizosaccharomyces pombe* について，過剰発現による増殖阻害を指標としたゲノム全体にわたる機能遺伝子の網羅的な探索手法を試みた。チアミンにより制御可能なプロモーターを用いて構築された遺伝子過剰発現株169株を用いて，固体培地上における遺伝子過剰発現状態のコロニー形成能を調べた結果，20株がコロニーを全く形成しなかった。次に，この20株について，遺伝子強制発現のシャットオフによる生育回復試験を行った。その結果，20株すべてが正常細胞と同程度に生育を回復した。従って，遺伝子過剰発現状態において全くコロニー形成しなかった20株は，死滅状態ではなく，増殖停止状態の細胞であると考えられた。次に，この20株について，細胞形態および細胞小器官（核，隔壁，アクチン）の形態と局在を調べた結果，種々の形態異常が観察された。このうち４遺伝子の機能については劣性変異株を用いて既に明らかにされている。これらの遺伝子機能は，本研究において遺伝子過剰発現により誘導された表現型から推測可能である。従って，過剰発現株を用いたゲノムワイドな網羅的探索手法は，従来の劣性変異株の表現型解析による機能遺伝子解析の代替手法となり得ると考えられる。実際，劣性変異で解析可能な遺伝子は，細胞増殖に必須とは限らない。以上の結果より，遺伝子過剰発現株を用いた優性遺伝学的な手法は，分裂酵母の機能遺伝子の網羅的探索手法として極めて有用であると考えられた。

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